Oligonucleotide length- and probe number-dependent assembly of gold nanoparticle on triangular DNA origami

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1. Materials

1.1 Abbreviations

TAE: tris acetate-EDTA buffer

BSPP: bis (p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt

TCEP: tris (2-carboxyethyl) phosphine hydrochloride

0.5×TBE: 89 mM tris, 89 mM boric acid, 2mM EDTA, pH=8.0

AAM: atomic force microscopy

NAS: nucleic acid stain

1.2 Chemicals

Single-stranded M13mp18 DNA was purchased from New England Biolabs (Catalog number: #N4040S). All staple strands and thoilated ssDNA were purchased from Shanghai Sangon Biotech Inc. (Shanghai, China). TAE was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Magnesium acetate tetrahydrate ($C_4H_6MgO_4•4H_2O$), sodium citrate tribasic dehydrate ($C_6H_5Na_3O_7·2H_2O$), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), chloroauric acid ($HAuCl_4$), sodium chloride were bought from Aladdin (Shanghai, China). Bis (p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BSPP) was obtained from Sigma-Aldrich (USA).

EDTA, Tris, boric acid, CH_3OH, nucleic acid stain (NAS) are of analytical reagent grade. All solutions were prepared with deionized water (≥18MΩ cm) generated from a Millipore Q water purification system.

1.3 Sequence of the staple ssDNA and thiolated oligonucleotides

DNA origami was prepared according to Rothemund's approach with minor modifications[1].

Staple name	(DNA sequences, from 5' to 3')
t11s18h,D1,	AATACTGCGGAATCGTAGGGGGTAATAGTAAAATGTTTAGACT
t11s28h,E1,	TCTTTGATTAGTAATAGTCTGTCCATCACGCAAATTAACCGTT
t11s8h,A1,	CAGAAGGAAACCGAGGTTTTTAAGAAAAGTAAGCAGATAGCCG
t1s10g,B2,	GACGGGAGAATTAACTCGGAATAAGTTTATTTCCAGCGCC
t1s12i,C2,	TCATATGTGTAATCGTAAAACTAGTCATTTTC
t1s14i,D2,	GTGAGAAAATGTGTAGGTAAAGATACAACTTT
t1s16i,E2,	GGCATCAAATTTGGGGCGCGAGCTAGTTAAAG
t1s18i,A2,	TTCGAGCTAAGACTTCAAATATCGGGAACGAG
t1s20g,G2,	GAATACCACATTCAACTTAAGAGGAAGCCCGATCAAAGCG
t1s22i,H2,	TCGGGAGATATACAGTAACAGTACAAATAATT
t1s24i,A3,	CCTGATTAAAGGAGCGGAATTATCTCGGCCTC
t1s26i,B3,	GCAAATCACCTCAATCAATATCTGCAGGTCGA
t1s28i,C3,	CGACCAGTACATTGGCAGATTCACCTGATTGC
t1s2i,D3,	CGGGGTTTCCTCAAGAGAAGGATTTTGAATTA
t1s30g,E3,	TTGACGAGCACGTATACTGAAATGGATTATTTAATAAAAG
t1s4i,A3,	AGCGTCATGTCTCTGAATTTACCGACTACCTT
t1s6i,G3,	TTCATAATCCCCTTATTAGCGTTTTTCTTACC
t1s8i,H3,	**ATGGTTTATGTCACAATCAATAGATATTAAAC**
t2s11g,A4,	AGAAAAGCCCCAAAAAGAGTCTGGAGCAAACAATCACCAT
t2s13g,B4,	ACAGTCAAAGAGAATCGATGAACGACCCCGGTTGATAATC
t2s15f,C4,	ATAGTAGTATGCAATGCCTGAGTAGGCCGGAG
t2s17f,D4,	AACCAGACGTTTAGCTATATTTTCTTCTACTA

t2s1g,E4, GATAAGTGCCGTCGAGCTGAAACATGAAAGTATACAGGAG

t2s21g,A4, CCTGATTGCTTTGAATTGCGTAGATTTTCAGGCATCAATA

t2s23g,G4, TGGCAATTTTTAACGTCAGATGAAAACAATAACGGATTCG

t2s25f,H4, AAGGAATTACAAAGAAACCACCAGTCAGATGA

t2s27f,A5, GGACATTCACCTCAAATATCAAACACAGTTGA

t2s3g,B5, TTTGATGATTAAGAGGCTGAGACTTGCTCAGTACCAGGCG

t2s5f,C5, CCGGAACCCAGAATGGAAAGCGCAACATGGCT

t2s7f,D5, AAAGACAACATTTTCGGTCATAGCCAAAATCA

t3s10g,E5, GTCAGAGGGTAATTGATGGCAACATATAAAAGCGATTGAG

t3s14e,A5, CAATATGACCCTCATATATTTTAAAGCATTAA

t3s16e,G5, CATCCAATAAATGGTCAATAACCTCGGAAGCA

t3s18g,H5, AACTCCAAGATTGCATCAAAAAGATAATGCAGATACATAA

t3s20g,A6, CGCCAAAAGGAATTACAGTCAGAAGCAAAGCGCAGGTCAG

t3s24e,B6, TAATCCTGATTATCATTTTGCGGAGAGGAAGG

t3s26e,C6, TTATCTAAAGCATCACCTTGCTGATGGCCAAC

t3s28g,D6, AGAGATAGTTTGACGCTCAATCGTACGTGCTTTCCTCGTT

t3s30g,E6, AGAATCAGAGCGGGAGATGGAAATACCTACATAACCCTTC

t3s4e,A6, TGTACTGGAAATCCTCATTAAAGCAGAGCCAC

t3s6e,G6, CACCGGAAAGCGCGTTTTCATCGGAAGGGCGA

t3s8g,H6, CATTCAACAAACGCAAAGACACCAGAACACCCTGAACAAA

t4s11g,A7, GCAAATATTTAAATTGAGATCTACAAAGGCTACTGATAAA

t4s13g,B7, CGTTCTAGTCAGGTCATTGCCTGACAGGAAGATTGTATAA

t4s15f,C7, CAGGCAAGATAAAAATTTTTAGAATATTCAAC

t4s17f,D7, GATTAGAGATTAGATACATTTCGCAAATCATA

t4s1g,E7, TAGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG

t4s21g,A7, GCGCAGAGGCGAATTAATTATTTGCACGTAAATTCTGAAT

t4s23g,G7, GATTATACACAGAAATAAAGAAATACCAAGTTACAAAATC

t4s25f,H7, TAGGAGCATAAAAGTTTGAGTAACATTGTTTG

t4s27f,A8, TGACCTGACAAATGAAAAATCTAAAATATCTT

t4s3g,B8, TTTAACGGTTCGGAACCTATTATTAGGGTTGATATAAGTA

t4s5f,C8, CTCAGAGCATATTCACAAACAAATTAATAAGT

t4s7f,D8, GGAGGGAATTTAGCGTCAGACTGTCCGCCTCC

t5s10g,E8, GATAACCCACAAGAATGTTAGCAAACGTAGAAAATTATTC

t5s14e,A8, TTAATGCCTTATTTCAACGCAAGGGCAAAGAA

t5s16e,G8, TTAGCAAATAGATTTAGTTTGACCAGTACCTT

t5s18g,H8, TAATTGCTTTACCCTGACTATTATGAGGCATAGTAAGAGC

t5s20g,A9, AACACTATCATAACCCATCAAAAATCAGGTCTCCTTTTGA

t5s24e,B9, AATGGAAGCGAACGTTATTAATTTCTAACAAC

t5s26e,C9, TAATAGATCGCTGAGAGCCAGCAGAAGCGTAA

t5s28g,D9, GAATACGTAACAGGAAAAACGCTCCTAAACAGGAGGCCGA

t5s30g,E9, TTAAAGGGATTTTAGATACCGCCAGCCATTGCGGCACAGA

t5s4e,A9, CCTTGAGTCAGACGATTGGCCTTGCGCCACCC

t5s6e,G9, TCAGAACCCAGAATCAAGTTTGCCGGTAAATA

t5s8g,H9, TTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGA

t6s15g,B10, ATAAAGCCTTTGCGGGAGAAGCCTGGAGAGGGTAG

t6s17f,C10, TAAGAGGTCAATTCTGCGAACGAGATTAAGCA

t6s25g,E10, TCAATAGATATTAAATCCTTTGCCGGTTAGAACCT

t6s27f,A10, CAATATTTGCCTGCAACAGTGCCATAGAGCCG

t6s5g,H10, CAGAGCCAGGAGGTTGAGGCAGGTAACAGTGCCCG

t6s7f,A11, ATTAAAGGCCGTAATCAGTAGCGAGCCACCCT

t7s10g,B11, ATAAGAGCAAGAAACATGGCATGATTAAGACTCCGACTTG

t7s14e,C11, ATGACCCTGTAATACTTCAGAGCA

t7s16e,D11, TAAAGCTATATAACAGTTGATTCCCATTTTTG

t7s18g,E11, CGGATGGCACGAGAATGACCATAATCGTTTACCAGACGAC

t7s20g,A11, GATAAAAACCAAAATATTAAACAGTTCAGAAATTAGAGCT

t7s24e,G11, ACAATTCGACAACTCGTAATACAT

t7s26e,H11, TTGAGGATGGTCAGTATTAACACCTTGAATGG

t7s28g,A12, CTATTAGTATATCCAGAACAATATCAGGAACGGTACGCCA

t7s30g,B12, GAATCCTGAGAAGTGTATCGGCCTTGCTGGTACTTTAATG

t7s4e,C12, GCCGCCAGCATTGACACCACCCTC

t7s6e,D12, AGAGCCGCACCATCGATAGCAGCATGAATTAT

t7s8g,E12, CACCGTCACCTTATTACGCAGTATTGAGTTAAGCCCAATA

t8s17g,G12, TAATTGCTTGGAAGTTTCATTCCAAATCGGTTGTA

t8s27g,A1, CGCGAACTAAAACAGAGGTGAGGCTTAGAAGTATT

t8s7g,C1, AGCCATTTAAACGTCACCAATGAACACCAGAACCA

t9s10h,D1, TATCTTACCGAAGCCCAAACGCAATAATAACGAAAATCACCAG

t9s16e,E1, ACTAAAGTACGGTGTCGAATATAA

t9s18g,A1, TGCTGTAGATCCCCCTCAAATGCTGCGAGAGGCTTTTGCA

t9s20h,G1, AAAGAAGTTTTGCCAGCATAAATATTCATTGACTCAACATGTT

t9s26e,H1, ACCACCAGCAGAAGATGATAGCCC

t9s28g,A2, TAAAACATTAGAAGAACTCAAACTTTTTATAATCAGTGAG

t9s30h,B2, GCCACCGAGTAAAAGAACATCACTTGCCTGAGCGCCATTAAAA

t9s6e,C2, CCATTAGCAAGGCCGGGGGAATTA

t9s8g,D2, GAGCCAGCGAATACCCAAAAGAACATGAAATAGCAATAGC

t-10s17h,E2, ACCAACCTAAAAAATCAACGTAACAAATAAATTGGGCTTGAGA

t-10s27h,A2, AACTCACATTATTGAGTGTTGTTCCAGAAACCGTCTATCAGGG

t-10s7h,G2, ACGACAATAAATCCCGACTTGCGGGAGATCCTGAATCTTACCA t-12s19h,C3, CCTGACGAGAAACACCAGAACGAGTAGGCTGCTCATTCAGTGA t-12s29h,D3, ACGTGGACTCCAACGTCAAAGGGCGAATTTGGAACAAGAGTCC t-12s9h,E3, TGCTATTTTGCACCCAGCTACAATTTTGTTTTGAAGCCTTAAA

t-1s10e,A3, **AGAGAATAACATAAAAACAGGGAAGCGCATTA**

t-1s12i,G3, AGGGATAGCTCAGAGCCACCACCCCATGTCAA

t-1s14e,H3, ATTTTCTGTCAGCGGAGTGAGAATACCGATAT

t-1s14i,A4, CAACAGTTTATGGGATTTTGCTAATCAAAAGG

t-1s16e,B4, ATTCGGTCTGCGGGATCGTCACCCGAAATCCG

t-1s16i,C4, GCCGCTTTGCTGAGGCTTGCAGGGGAAAAGGT

t-1s18g,D4, CGACCTGCGGTCAATCATAAGGGAACGGAACAACATTATT

t-1s18i,E4, GCGCAGACTCCATGTTACTTAGCCCGTTTTAA

t-1s20e,A4, ACAGGTAGAAAGATTCATCAGTTGAGATTTAG

t-1s22i,G4, CGCGTCTGATAGGAACGCCATCAACTTTTACA

t-1s24e,H4, CAGTTTGACGCACTCCAGCCAGCTAAACGACG t-

1s24i,A5, AGGAAGATGGGGACGACGACAGTAATCATATT t-

1s26e,B5, GCCAGTGCGATCCCCGGGTACCGAGTTTTTCT

t-1s26i,C5, CTCTAGAGCAAGCTTGCATGCCTGGTCAGTTG

t-1s28g,D5, TTTCACCAGCCTGGCCCTGAGAGAAAGCCGGCGAACGTGG

t-1s28i,E5, CCTTCACCGTGAGACGGGCAACAGCAGTCACA

t-1s2i,A5, CCTTTTTTCATTTAACAATTTCATAGGATTAG

t-1s30e,G5, CGAGAAAGGAAGGGAAGCGTACTATGGTTGCT

t-1s4e,H5, TTATCAAACCGGCTTAGGTTGGGTAAGCCTGT

t-1s4i,A6, TTTAACCTATCATAGGTCTGAGAGTTCCAGTA

t-1s6e,B6, TTAGTATCGCCAACGCTCAACAGTCGGCTGTC

t-1s6i,C6, AGTATAAAATATGCGTTATACAAAGCCATCTT

t-1s8g,D6, TTTCCTTAGCACTCATCGAGAACAATAGCAGCCTTTACAG

t-1s8i,E6, **CAAGTACCTCATTCCAAGAACGGGAAATTCAT**

t-2s11g,A6, CCTCAGAACCGCCACCCAAGCCCAATAGGAACGTAAATGA

t-2s13g,G6, AGACGTTACCATGTACCGTAACACCCCTCAGAACCGCCAC

t-2s15f,H6, CACGCATAAGAAAGGAACAACTAAGTCTTTCC

t-2s17f,A7, ATTGTGTCTCAGCAGCGAAAGACACCATCGCC

t-2s1g,B7, AAAACAAAATTAATTAAATGGAAACAGTACATTAGTGAAT

t-2s21g,C7, GCTCATTTTTTAACCAGCCTTCCTGTAGCCAGGCATCTGC

t-2s23g,D7, GTAACCGTCTTTCATCAACATTAAAATTTTTGTTAAATCA

t-2s25f,E7, ACGTTGTATTCCGGCACCGCTTCTGGCGCATC

t-2s27f,A7, CCAGGGTGGCTCGAATTCGTAATCCAGTCACG

t-2s3g,G7, AGAGTCAAAAATCAATATATGTGATGAAACAAACATCAAG

t-2s5f,H7, ACTAGAAATATATAACTATATGTACGCTGAGA

t-2s7f,A8, TCAATAATAGGGCTTAATTGAGAATCATAATT

t-3s10g,B8, AACGTCAAAAATGAAAAGCAAGCCGTTTTTATGAAACCAA

t-3s14e,C8, GTTTTGTCAGGAATTGCGAATAATCCGACAAT

t-3s16e,D8, GACAACAAGCATCGGAACGAGGGTGAGATTTG

t-3s18g,E8, TATCATCGTTGAAAGAGGACAGATGGAAGAAAAATCTACG

t-3s20g,A8, TTAATAAAACGAACTAACCGAACTGACCAACTCCTGATAA

t-3s24e,G8, TGTAGATGGGTGCCGGAAACCAGGAACGCCAG

t-3s26e,H8, GGTTTTCCATGGTCATAGCTGTTTGAGAGGCG

t-3s28g,A9, GTTTGCGTCACGCTGGTTTGCCCCAAGGGAGCCCCCGATT

t-3s30g,B9, TAGAGCTTGACGGGGAGTTGCAGCAAGCGGTCATTGGGCG

t-3s4e,C9, GATTAAGAAATGCTGATGCAAATCAGAATAAA

t-3s6e,D9, CACCGGAATCGCCATATTTAACAAAATTTACG

t-3s8g,E9, AGCATGTATTTCATCGTAGGAATCAAACGATTTTTTGTTT

t-4s11g,A9, AGGTTTAGTACCGCCATGAGTTTCGTCACCAGGATCTAAA

t-4s13g,G9, AGCGTAACTACAAACTACAACGCCTATCACCGTACTCAGG

t-4s15f,H9, TAGTTGCGAATTTTTTCACGTTGATCATAGTT

t-4s17f,A10, GTACAACGAGCAACGGCTACAGAGGATACCGA

t-4s1g,B10, GAGCAAAAGAAGATGAGTGAATAACCTTGCTTATAGCTTA t-

4s21g,C10, GTTAAAATTCGCATTAATGTGAGCGAGTAACACACGTTGG t-

4s23g,D10, GGATAGGTACCCGTCGGATTCTCCTAAACGTTAATATTTT

t-4s25f,E10, AGTTGGGTCAAAGCGCCATTCGCCCCGTAATG

t-4s27f,A10, CGCGCGGGCCTGTGTGAAATTGTTGGCGATTA

t-4s3g,G10, ACATAGCGCTGTAAATCGTCGCTATTCATTTCAATTACCT

t-4s5f,H10, GTTAAATACAATCGCAAGACAAAGCCTTGAAA

t-4s7f,A11, CCCATCCTCGCCAACATGTAATTTAATAAGGC

t-5s10g,B11, TCCCAATCCAAATAAGATTACCGCGCCCAATAAATAATAT

t-5s16e,D11, AACAGCTTGCTTTGAGGACTAAAGCGATTATA

t-5s18g,E11, CCAAGCGCAGGCGCATAGGCTGGCAGAACTGGCTCATTAT

t-5s20g,A11, ACCAGTCAGGACGTTGGAACGGTGTACAGACCGAAACAAA

t-5s26e,H11, TGCTGCAAATCCGCTCACAATTCCCAGCTGCA

t-5s28g,A12, TTAATGAAGTTTGATGGTGGTTCCGAGGTGCCGTAAAGCA

t-5s30g,C12, CTAAATCGGAACCCTAAGCAGGCGAAAATCCTTCGGCCAA

t-5s6e,D12, GTGTGATAAGGCAGAGGCATTTTCAGTCCTGA

t-5s8g,E12, ACAAGAAAGCAAGCAAATCAGATAACAGCCATATTATTTA

t-6s13f,A12, ACAGACAGCCCAAATCTCCAAAAAAAAATTTCTTA

t-6s15c,G12, CGAGGTGAGGCTCCAAAAGGAGCC

t-6s17f,H12, ACCCCCAGACTTTTTCATGAGGAACTTGCTTT

t-6s23f,A1, CGGCGGATTGAATTCAGGCTGCGCAACGGGGGATG

t-6s25c,B1, TGGCGAAATGTTGGGAAGGGCGAT

t-6s27f,C1, TGTCGTGCACACAACATACGAGCCACGCCAGC

t-6s3f,D1, TCCCTTAGAATAACGCGAGAAAACTTTTACCGACC

t-6s5c,E1, GTTTGAAATTCAAATATATTTTAG

t-6s7f,A1, AATAGATAGAGCCAGTAATAAGAGATTTAATG

t-7s10g,G1, GCCAGTTACAAAATAATAGAAGGCTTATCCGGTTATCAAC t-7s18g,A2, AAAACACTTAATCTTGACAAGAACTTAATCATTGTGAATT t-7s20g,B2, ACCTTATGCGATTTTATGACCTTCATCAAGAGCATCTTTG

t-7s28g,D2, TTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAAT

t-7s30g,E2, CAAGTTTTTTGGGGTCGAAATCGGCAAAATCCGGGAAACC

t-7s8g,G2, GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTT

t-7s8g,G2, GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTT

t-8s15f,H2, CGGTTTATCAGGTTTCCATTAAACGGGAATACACT

t-8s17c,A3, GGCAAAAGTAAAATACGTAATGCC

t-8s25f,B3, TCTTCGCTATTGGAAGCATAAAGTGTATGCCCGCT

t-8s27c,C3, GCGCTCACAAGCCTGGGGTGCCTA

t-8s5f,D3, TTCTGACCTAAAATATAAAGTACCGACTGCAGAAC

t-8s7c,E3, TCAGCTAAAAAAGGTAAAGTAATT

t-9s10g,A3, ACGCTAACGAGCGTCTGGCGTTTTAGCGAACCCAACATGT

t-9s20g,H3, TGGTTTAATTTCAACTCGGATATTCATTACCCACGAAAGA

t-9s30g,B4, CGATGGCCCACTACGTATAGCCCGAGATAGGGATTGCGTT

ts-rem1, D4, GCGCTTAATGCGCCGCTACAGGGC

t-5s2e-t6s23c-3T,A6, TTAATTAATTTTTTACCATATCAAA

t-7s4e-t8s25c-2T,B6, TTAATTTCATCTTAGACTTTACAA

t-9s6e-t10s27c-1T,C6, CTGTCCAGACGTATACCGAACGA

t-11s8e-t12s29c-0T,D6, TCAAGATTAGTGTAGCAATACT

t-5s12e-t6s3c-3T,E6, TGTAGCATTCCTTTTATAAACAGTT

t-7s14e-t8s5c-2T,A6, TTTAATTGTATTTCCACCAGAGCC

t-9s16e-t10s7c-1T,G6, ACTACGAAGGCTTAGCACCATTA

t-11s18e-t12s9c-0T,H6, ATAAGGCTTGCAACAAAGTTAC

t-5s22e-t6s13c-3T,A7, GTGGGAACAAATTTCTATTTTTGAG

t-7s24e-t8s15c-2T,B7, CGGTGCGGGCCTTCCAAAAACATT

t-9s26e-t10s17c-1T,C7, ATGAGTGAGCTTTTAAATATGCA

t-11s28e-t12s19c-0T,D7, ACTATTAAAGAGGATAGCGTCC

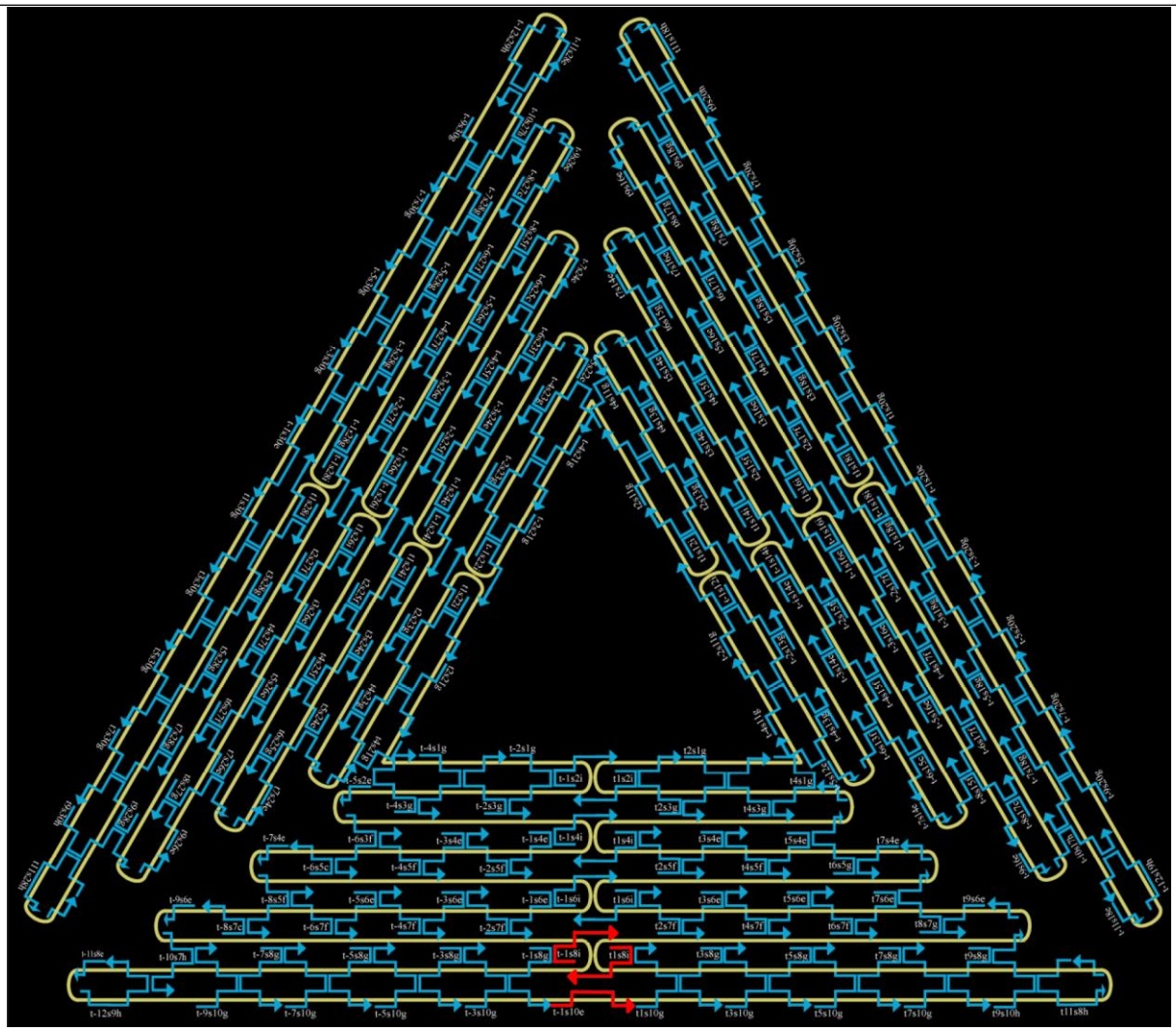

Fig. S1 Locations of the three probes on the triangular origami DNA. The modified staple ssDNA molecules were red-colored in the image.

As to the DNA origami with probes, the blue colored strands were modified:

- **In the first set of experiments：**

A 60-mer joint strand was stretched out from the DNA origami substrate. In the experiments, the staple ssDNA t-1s10e,F3 was modified as below:

AGAGAATAACATAAAAACAGGGAAGCGCATTA**TTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTGTTTGGTTGGTGTGGTTGG**（From 5’ to 3’）.

The red part with 60-mer length was added to the 3’ end of the staple ssDNA.

AuNPs were immobilized with the following thiolated ssDNA, respectively. The red part was served as steric hindrance for combination of AuNPs on DNA origami.

20-mer thiolated-ssDNA (From 5’ to 3’)：

SH-**TTTTTT**CCAACCACACCAACCAAACA

40-mer thiolated-ssDNA (From 5’ to 3’)：

SH-**TTTTTT**CCAACCACACCAACCAAACAAAAAAAAAAAAAAAAAAAA

60-mer thiolated-ssDNA (From 5’ to 3’)：

SH-**TTTTTT**CCAACCACACCAACCAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

- **In the second set of experiments：**

A 20-mer joint strand was stretched out from the DNA origami substrate. In the experiments, the staple ssDNA t-1s10e,F3 was modified as below:

AGAGAATAACATAAAAACAGGGAAGCGCATTATGTTTGGTTGGTGTGGTTGG（From 5’ to 3’）. The red part with 20-mer length was added to the 3’ end of the staple ssDNA.

A 40-mer joint strand was stretched out from the DNA origami substrate. In the experiments, the staple ssDNA t-1s10e,F3 was modified as below:

AGAGAATAACATAAAAACAGGGAAGCGCATTATTTTTTTTTTTTTTTTTTTTTGTTTGGT TGGTGTGGTTGG（From 5’ to 3’）. The red part with 40-mer length was added to the 3’ end of the staple ssDNA.

A 60-mer joint strand was stretched out from the DNA origami substrate. In the experiments, the staple ssDNA t-1s10e,F3 was modified as below:
AGAGAATAACATAAAAACAGGGAAGCGCATTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTTTGGTTGGTGTGGTTGG（From 5’ to 3’）.The red part with 60-mer length was added to the 3’ end of the staple ssDNA.

AuNPs were immobilized with the 20-mer ssDNA. The red part was served as steric hindrance for combination of AuNPs on DNA origami.
SH-TTTTTTCCAACCACACCAACCAAACA (From 5’ to 3’)

- **In the third set of experiments:**

Three 20-mer joint strands were stretched out from the DNA origami substrate. In the experiments, the staple ssDNA t-1s10e,F3, t-1s8i, H3 and t-1s8i, E6 were modified, respectively, as below:

Probe # 1: (t-1s10e,F3)

AGAGAATAACATAAAAACAGGGAAGCGCATTATGTTTGGTTGGTGTGGTTGG（From 5’ to 3’）. The red part with 60-mer length was added to the 3’ end of the staple ssDNA.

Probe # 2: (t-1s8i, H3)

ATGGTTTATGTCACAATCAATAGATATTAAACTGTTTGGTTGGTGTGGTTGG（From 5’ to 3’）.

Probe # 3: (t-1s8i, E6)

CAAGTACCTCATTCCAAGAACGGGAAATTCATTGTTTGGTTGGTGTGGTTGG（From 5’ to 3’）.

AuNPs were modified with 20-mer thiolated oligonucleotides：

SH-TTTTTTCCAACCACACCAACCAAACA (From 5’ to 3’)

2. Preparation of DNA origami

The single-strand M13mp18 virus DNA and the staple strands (including the modified ones) were mixed in a molar ratio of 1:10 in the 1×TAE~Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, 12.5 mM magnesium acetate). The mixture was annealed from 95°C to 20°C in a PCR instrument (1℃/100s). [1] The products were filtered with centrifugal filters using the washing buffer (1×TAE~Mg^{2+} buffer) for three times to remove excess staple strands.[2]

3. Modification of AuNPs

AuNP-DNA conjugates were synthesized according to Ding's route. [3] Briefly, 15 mg BSPP was dissolved in a 50 mL AuNPs solution, followed by a stirring in dark at room temperature for 12 hours. Solid sodium chloride was added in slowly until the color of the solution changes from wine red to lilac. The products were collected by centrifugation at 10000 rpm for 15 min and re-suspended in a mixture of BSPP and CH_3OH. After a second round of centrifugation, the AuNPs were suspended in 1mL BSPP (2.5mM). Thiolated oligonucleotides were activated with TCEP (10mM) for several hours and then incubated with the AuNPs at a molar ratio of 200:1 in a 0.5× TBE buffer containing 50 mM NaCl for 40 hours at room temperature. After washing with the buffer for three times, the oligonucleotides-conjugated AuNPs were harvested via centrifugation. The UV-vis spectra of the final products were measured to determine the concentration according to Wolfgang Haiss's method. [4]

4. Assembly of AuNPs with the modified DNA origami templates

The conjugated AuNPs were mixed with the modified DNA origami templates. The mixture was cooled from 43 to 20 °C to allow the hybridization of the complementary ssDNA for the assembly of AuNPs with the modified DNA origami templates. [3]

5. AFM characterization

A freshly cleaved mica was pre-treated with a $MgCl_2$ solution. The adsorbed Mg^{2+} ions could facilitate the attachment of negatively charged DNA origami and AuNPs. Then, 10 μL sample were dropped on the mica surface, drying in air for 6 hours.[5] The AAM images were scanned with a Dimension Icon scanning probe microscope (Bruker, USA) at ambient conditions.

6. Gel electrophoresis

The resulting DNA origami-AuNPs were loaded on 1.0% agarose gels with (5 μL NAS) and run for three hours at 100V. [6] [7] The gel images were photographed using a digital camera system (Chemi XR5).

7. Data analysis

Data were collected from at least 4 runs of independent experiments. Differences between different groups were tested for significance using Student's t test (Origin 6.0).

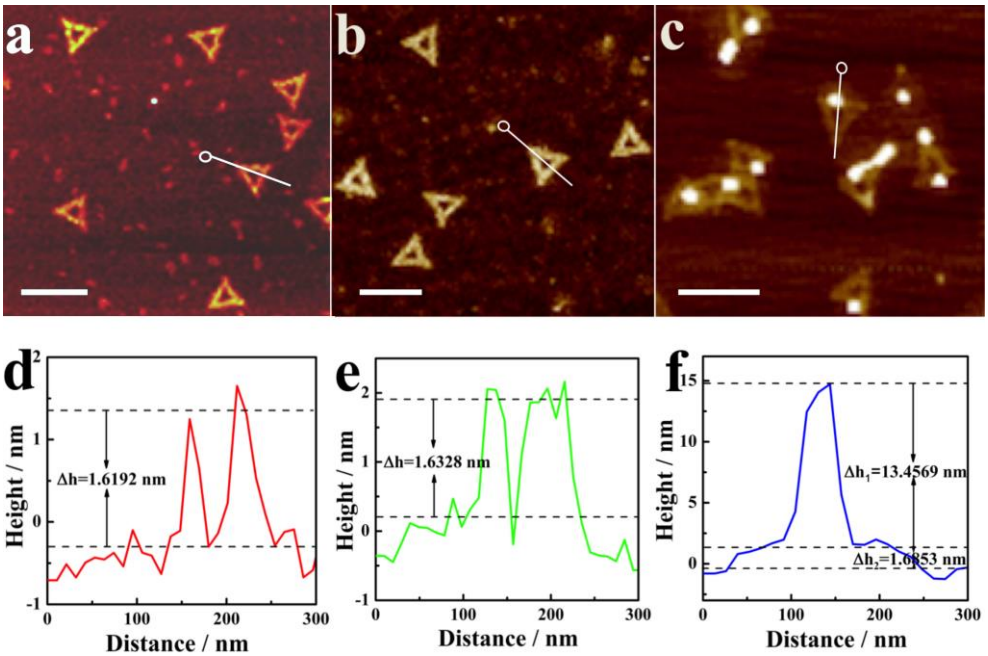

Fig. S2 AAM images of (a) DNA origami triangles, (b) DNA origami triangles modified with a single strand DNA probe and (c) AuNP-attached DNA origami triangles. Height profiles of (d) a DNA origami triangle, (e) a DNA origami triangle modified with a single strand DNA probe and (f) a AuNP-attached DNA origami triangle.

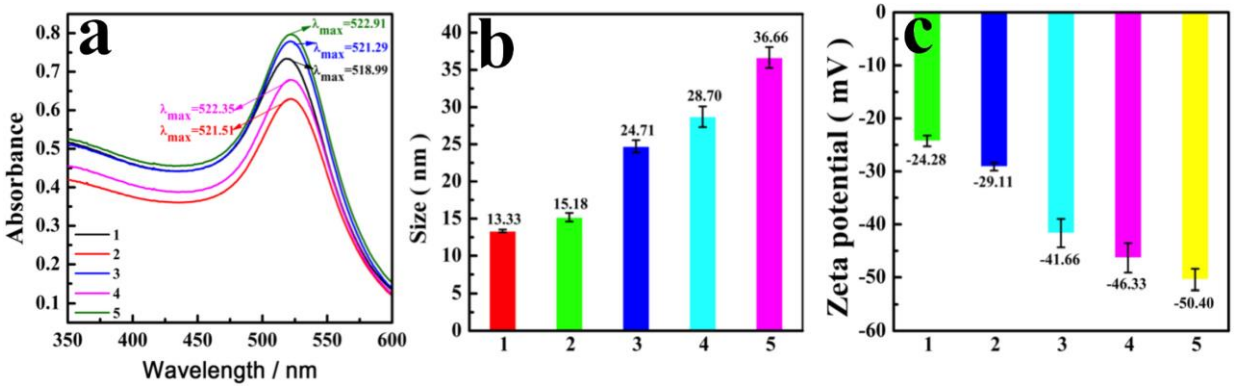

Fig. S3 UV-vis spectra (a), hydrodynamic size (b) and zeta potential (c) of AuNPs before and after modification with BSPP and ssDNA. Data in (b) and (c) are presented as the average ± standard deviation from three independent measurements. The numbers 1, 2, 3, 4 and 5 correspond to AuNPs, BSPP-AuNPs, 20-mer DNA-BSPP-AuNPs, 40-mer DNA-BSPP-AuNPs and 60-mer DNA-BSPP-AuNPs, respectively.

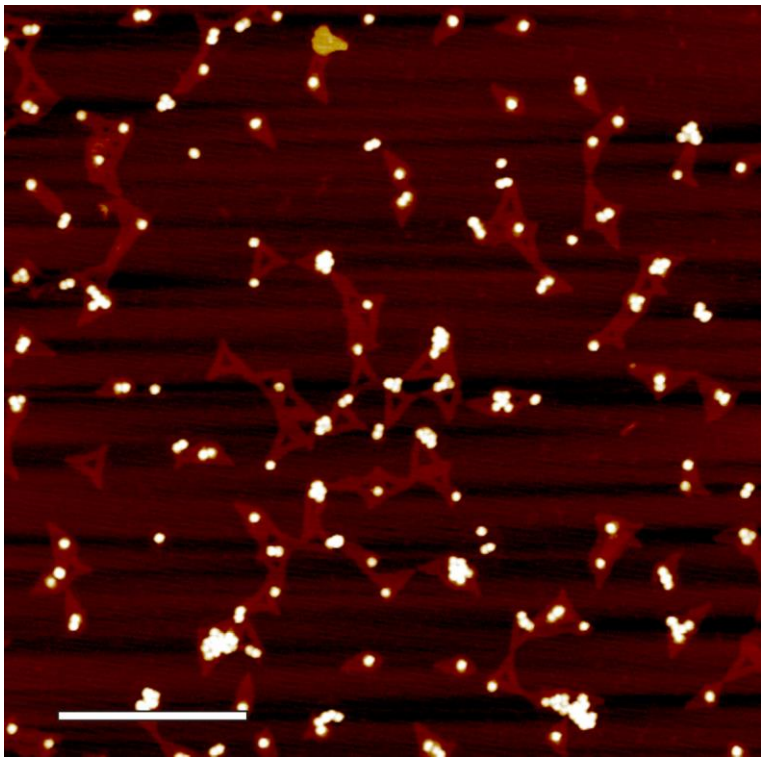

Fig. S4 AAM image of the DNA origami attached 40-mer DNA-BSPP-AuNPs.

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